Functional Amphiphilic and Bolaamphiphilic Polydiacetylene Assemblies with Controlled Optical and Morphological Properties

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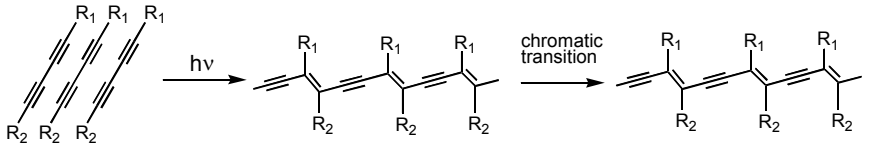
Amino acid-terminated amphiphilic and bolaamphiphilic diacetylene lipids were synthesized and assembled to form microstructures of varied morphologies. UV irradiation of the assemblies leads to conjugated polymers with unique optical properties. Chromatic transition of polydiacetylene materials in response to pH and thermal effect and their morphological transformation upon lipid doping are discussed.

Conjugated polymers capable of responding to external stimuli by changes in optical, electrical or electrochemical properties are of great interest for the design of various sensors.[1-3] They are attractive materials for constructing direct sensing devices because the signal transducer element (the conjugation system of the polymer) and molecular recognition moiety can potentially be built within a single unit, rendering them amenable to microfabrication. Polydiacetylenes (PDAs) represent one of the most promising chemical platforms for sensors, especially with colorimetric detection of analytes. The delocalized electronic

structure allows strong absorption in the UV-visible range, giving the material a blue appearance. The optical properties of PDA can be dramatically altered from blue to red by external stimuli such as heat, organic solvent, pH and mechanical stress (see scheme below). A particularly interesting stimulus is the binding of biological analytes at the polymer-media interface. The recognition



blue phase red phase

of a ligand by a membrane-associated receptor or an enzyme (covalently or non-covalently incorporated into the PDA scaffold) provides the needed driving force to induce chromatic transition of PDA upon the occurrence of the interfacial binding event (i.e., biochromism), leading to the birth of colorimetric biosensors for influenza virus, bacterial toxins and *E. coli*.[4-6]

While the mechanistic detail of chromic shifts of PDAs in response to various environmental perturbations has been extensively investigated,[7-9] the design of functional PDAs with controlled supramolecular structures and customized optical properties is still in its infancy. PDA as a sensing material has limitations, particularly in detection sensitivity, processability and durability. New chemistries allowing for modification of the PDA systems should focus on improvements in these areas. For instance, headgroup derivatization can affect the original color of the polymer in the coplanar conformation of the ene-yne conjugation backbone, and therefore determine the type of chromic transition occurring upon the departure from coplanarity.[10] Attachment of an ionic molecule as headgroup could provide a useful means to alter the surface charge distribution and hydrophobicity around the recognition interface, striking a delicate balance between repulsive steric interactions and attractive *van der Waals* interactions, and thereby offering the possibility to optimize the chromatic transition properties.[11,12] More effective approaches involve alteration of hydrophobic lipid core length and the replacement of amphiphilic diacetylene lipids with a bolaamphiphilic lipid. Strengthening of hydrogen bonding interactions on both faces of the transmembranic structures could enhance the crystallinity of the lipid packing arrangement and possibly lead to the formation of novel microstructures.[13,14]

Much remains to be learned before the true rational design of functional PDAs can be realized, as either the transducer of chemical and biological colorimetric sensors or the molecular template of functional composites and devices with structural control ranging from nanoscopic to microscopic levels. In this chapter, we will discuss the design and preparation of mono-functional (amphiphilic) and bis-functional (bolaamphiphilic) PDAs that adopt exotic

morphologies but retain characteristic optical properties that are commonly observed with conventional Langmuir-Blodgett film[15,16] and vesicular[8,17] PDA assemblies. Emphasis will be placed on the derivatization of PDA templates with various amino acids. We will demonstrate that morphological transformations from extended helical ribbons to organized nanofibers, along with chromatic transition, can be manipulated via pH control. Transformations from ribbons to vesicular structures by doping with ganglioside G_{M1} and lipophilic cholesterol in a controlled manner will also be discussed.

I. Amino Acid-Terminated Amphiphilic Polydiacetylenes

Monomer synthesis, microstructure formation and characterization

Modification of amphiphilic diacetylene lipids with a series of naturally occurring amino acids is straightforward. 10,12-Pentacosadiynoic acid was converted to a succinimidyl ester in the presence of N-hydroxysuccinimide and EDC, and then coupled with the N-terminus of corresponding amino acids in a THF/H_2O mixed solvent to yield the derivatized lipids through an amide linkage (Figure 1). The choice of amino acids as headgroup is to create a compatible surface on microstructures for protein-related sensing applications. In addition,

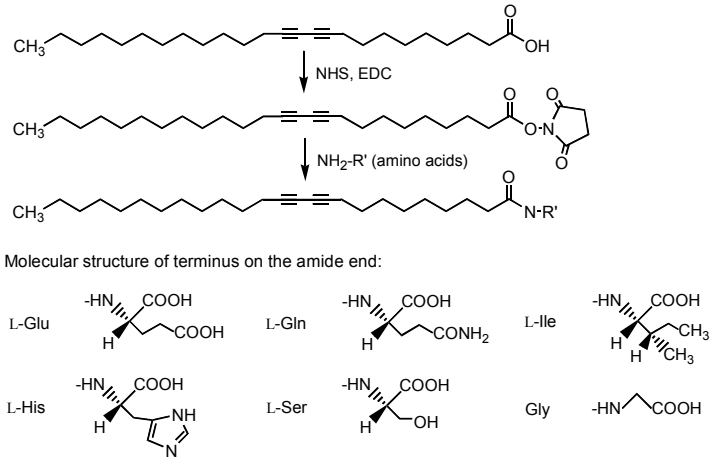


Figure 1. Synthetic scheme and molecular structures of amino acid terminated diacetylene lipids.

these optically pure amino acids vary in polarity, allowing surface charge and hydrophilicity to be manipulated in a broad range and controllable manner. To obtain lipid microstructures, dried lipid was dissolved in methanol to which

warm deionized water was added dropwise under vigorous stirring. Prior to polymerization and colorimetric characterization, the sample was dialyzed against water using a Spectra/Por membrane tubing to remove residual methanol. Lipid bilayer vesicles were obtained by hydration of diacetylenic lipids using probe sonication. Photopolymerization of diacetylene microstructures and bilayer vesicles was realized by UV irradiation at 254 nm.

Figure 2 shows the TEM images of microstructures made from amino acid terminated diacetylene lipids. For L-Glu-PDA (Fig. 2A), the aggregate consists of twisted and untwisted ribbons and fibers, with their lengths varying from

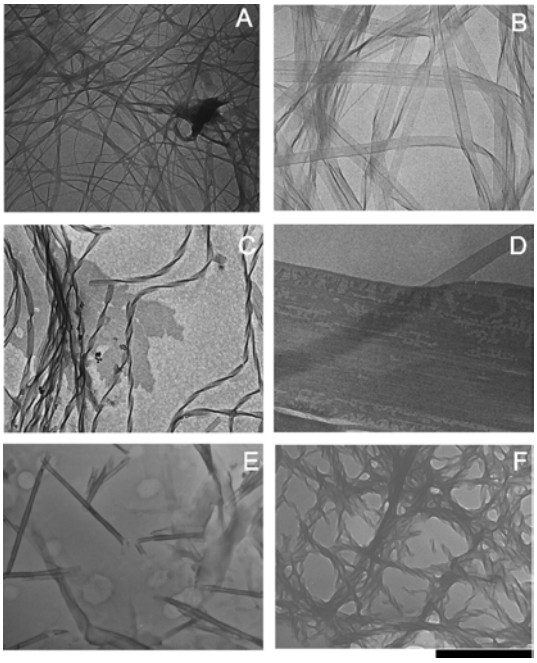


Figure 2. TEM images of microstructures made from amino acid terminated diacetylene lipids. (A) L-Glu-PDA, (B) L-Gln-PDA, (C) L-His-PDA, (D) Gly-PDA, (E) L-Ser-PDA and (F) L-Ile-PDA. The bar is 0.6 μm for (A), (B) and (F); 0.8 μm for (C) & (D); 2 μm for (E).

several to hundreds of microns. Under UV irradiation, L-Glu-PDA microstructures readily polymerize to give a dark blue color. L-Gln-PDA lipid forms similar ribbon shaped microstructures (Fig. 2B). However, the ribbon assemblies are more uniform in size. The typical ribbon width and thickness is around 150 nm and 8 nm, respectively. For L-His-PDA, the formation of extended (up to several microns) helical assemblies was readily observed (Fig.

3C), with right-handed helical twists averaging 60 nm in diameter. A large amount of planar platelets coexist with the helices. To verify the headgroup chirality effect on helical microstructure formation, achiral Gly-PDA lipid was used as a negative control. As expected, large amount of flat sheets and platelets without apparent twisting or curvature was obtained (Fig. 2D). L-Ser-PDA forms open tubular assemblies (Fig. 2E). The average diameter of the tubules is around 0.2 μm, with an estimated tubular wall thickness around 20 nm. The coexistence of a significant amount of sheets in the sample, especially the layers wrapped around the open end of the tubules, suggests that the formation of L-Ser-PDA tubules is through a rolling up mechanism.[18] L-Ile-PDA lipids, on the other hand, form networks of highly twisted braided ribbons (Fig. 2F). It is worth mentioning that twisted ribbons are the exclusive morphology observed with the L-Ile-PDA assembly.

The relationship between molecular structure and the preferential morphology of a supramolecular assembly is poorly understood. Extensive experimental studies indicated that chirality, conformation and hydrogen bonding forming ability of the polar headgroup of a lipid amphiphile are determining factors. For all the microstructures studied here, hydrogen bonding exists extensively, with an especially high degree in L-Glu-PDA assembly. The TEM results here seem to concur that headgroup chirality and the balance of headgroup size, polarity, and the degree of favorable interactions (e.g. H-bonding), in addition to the balance of dipolar forces,[19] are critical to the rise of lipid bilayer curvatures.

Colorimetric properties of amphiphilic PDA microstructures

The polymerization of the organized diacetylene lipid assemblies is a topochemical process, requiring optimal packing of the diacetylenic segments to allow propagation of an extended ene-yne conjugation backbone. The conjugated polymer absorbs light strongly around 650 nm, giving the material a blue appearance. Considerable investigations have been recently reported on the chromism of PDA bilayer vesicles and LB thin films,[10] in the light of using PDA assemblies for colorimetric sensors. Diacetylene lipid microstructures are structurally similar to their bilayer or monolayer counterparts and possess the intrinsic features as organized assemblies that should allow topochemical reaction and polymerization.

It is worth noting that all the amino acid-diacetylene lipid microstructures studied here could be polymerizable to form blue colored PDAs. However, only hydrophilic amino acid lipids can readily form bilayer vesicles and allow polymerization.[11] The intensity of the initial blue color, however, varies with headgroups. Amino acids with hydrophilic segments give the darkest blue appearance, while hydrophobic amino acids (Ile-) produce barely noticeable blue appearance.

The colorimetric properties of amino acid terminated PDAs were investigated by thermochromism and solution pH induced chromism. Quantitative analysis of chromatic transition was conducted by analyzing the colorimetric response (CR) as a function of solution pH.[16] The CR is defined as the percent change in the maximum adsorption at 646 nm with respect to the total absorption at 542 nm and 646 nm. Figure 3 shows the CR vs. pH for the amino acid terminated PDA microstructures. Sigmoidal curves were obtained

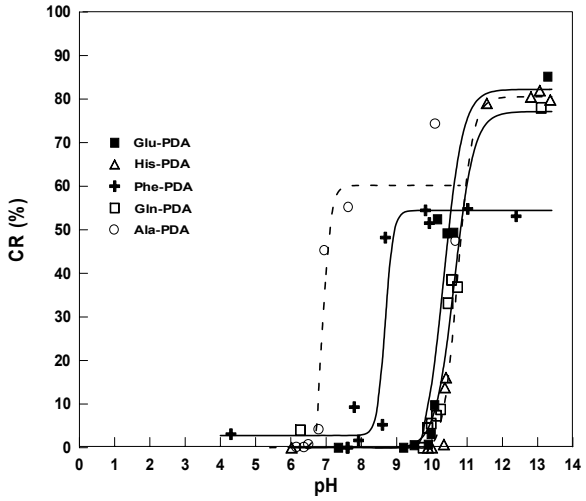


Figure 3. Colorimetric response for the polymerized microstructures as a function of solution pH.

for all microstructures studied, indicating sharp transitions from blue to red color upon pH increase. The transition point is defined by the CR_{50} values, namely the pH required to achieve 50% of the maximal color transition.[11] The CR_{50} values for "hydrophilic" amino acids (Glu, Gln and His) all fall between pH 10 to 11. For comparison, response curves for two "hydrophobic"amino acid lipids, Phe-PDA and Ala-PDA, are shown in Figure 3. The values for Ala and Phe are much lower (7.1 and 8.6, respectively). The "base-resistant" nature for "hydrophilic" amino acids differs from that obtained with the amino acid terminated PDA bilayer vesicles, where L-Glu-PDA was found to be the most base-sensitive. The color change of PDA microstructures can also be achieved by thermal treatment (thermochromism), as well documented in literature. Similar sigmoidal curves were obtained for amino acid terminated PDA lipids where L-Gln-PDA microstructure is the most heat resistant. A temperature as high as 71^{o}C is needed for the assembly to convert 50% of its color. Thermochromism of bilayer vesicles formed by amino acid terminated PDA lipids was also studied. Contrary to microstructures, the trend for vesicles seems

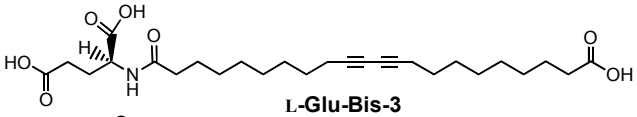
totally reversed. L-Gln-PDA vesicles are the most thermal sensitive while L-Glu-PDA is the least.

It has to be pointed out that thermochromism and pH induced chromism of PDA microstructures appear to proceed through different mechanisms. The temperature-induced side chain conformation transition is responsible for the thermochromism of the PDA microstructures. For pH induced chromatic transition, the headgroup undergoes significant reorganization as a result of ionization, causing a new conformational adjustment (staggered packing) that thereby imposes strains to the backbone. This has been further confirmed by FTIR studies on PDA microstructures. [12]

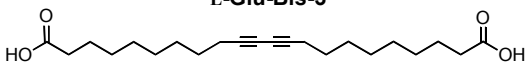
II. Bolaamphiphilic Polydiacetylenes

Amino acid-terminated bolaamphiphilic diacetylene lipid

The formation of a robust supramolecular assembly can be achieved through the deliberate installation of various functionalities throughout the molecular architecture that enforce the intermolecular association between assembling units. We designed an *L*-glutamic acid derivatized wedge-shaped bolaamphiphilic diacetylene lipid *L*-Glu-Bis-3 (structure seen below) as the



L-Glu-Bis-3



Bis-1

self-assembling unit of a highly organized molecular architecture. Compared to their amphiphilic lipid counterparts, bolaamphiphiles tend to form well-organized systems under very mild conditions. They mimic transmembranic lipids that some microorganisms synthesize for stabilizing membrane structures in response to extreme pH and temperature[20]. *L*-Glutamic acid residue attached to one end of 10,12-docosadiynedioic acid (Bis-1), along with the free carboxylate on the other end of the lipid, is designed to enhance favorable H-bonding interactions on the polar faces of the assembly. The diacetylene unit was placed at the center of the molecule to maximize the chance of proper alignment of polymerization units in different packing arrangements.

The synthesis of *L*-Glu-Bis-3 was reported elsewhere.[13] One terminal of Bis-1 was activated with N-hydroxysuccinimide before it was coupled with *L*-glutamic acid through an amide linkage, giving an overall 61% yield. Alternatively, activation of both carboxylate groups before the attachment of a

glutamate residue and the hydrolysis of the unreacted ester terminal could lead to an improved overall yield.

The self-assembling of *L*-Glu-Bis-3 occurred rapidly under mild conditions. Instead of probe sonication and subsequent low temperature incubation that are commonly required for amphiphilic lipids, vortexing and room temperature incubation was sufficient to ensure the formation of a stable for *L*-Glu-Bis-3 supramolecular assembly in aqueous media. UV-irradiation of the assembled material resulted in instantaneous polymerization of *L*-Glu-Bis-3, affording the material an intense blue appearance. The rapid polymerization indicates a highly ordered packing arrangement and the good alignment of diacetylene units.

Morphology and surface packing arrangement of bolaamphiphilic PDA

The morphology and surface packing arrangement of the polymer was characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM). TEM micrographs revealed the formation of ribbons tens of microns long (Figure 4). These ribbons are either flat or twisted with various

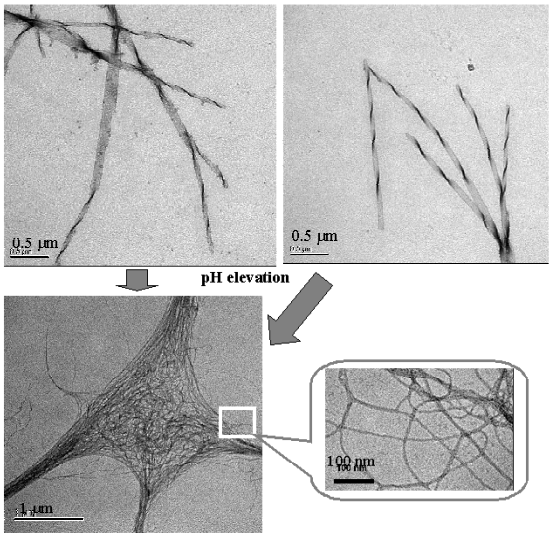


*Figure 4. TEM images of **poly-L-Glu-Bis-3** microstructures. The top images were obtained at pH 5.8 (D.I. water) showing rupture that is indicative of the origination of helices (top, left) and right-handedness of the twist (top, right). Lower images show structural transformation into nanofibers upon treatment of above microstructures with pH 7.5 Tris buffer.*

degrees of right-handed helicity, consistent with the observation of helical ribbons formed by *L*-amino acid terminated amphiphilic PDAs. Strips of parallel

domains were clearly visible on wider ribbons, apparently parallel with the propagation of the polymer backbone. These ribbons are 5 to 10 nm thick, corresponding to either a monolayer or a double layer lipid stacking. The widths of the ribbons vary from tens to hundreds of nanometers, with generally wider dimension for flat structures.

Among the many experimental discussions[21,22] and theoretical treatments[23,24] aiming at the explanation of tubular or helical lipid assembly formation, the chiral packing theory has been the cornerstone. It has been postulated that when bilayer chiral lipid amphiphiles aggregate, they first form wide sheets with sharply separated domains,[21] which would then break up along the domain edge to form narrower ribbons that are free to twist into helices, driven by chiral packing effect. Helical ribbons may further fuse into tubular structures to reduce edge energy. Our TEM data provides direct evidence to support this theory in the context of chiral bolaamphiphiles. The micrographs shown in Figure 4 captured the initiation of the transition from flat strips to helical ribbons through the rupturing of wider flat ribbons along the parallel domain edges. The narrower strips could then continue to twist into helical structures as a result of these chiral bolaamphiphiles' cumulative tilt away from the local surface normal. Formation of tubular structures, as observed at certain regions, is evidence of further winding of the helical ribbons to reduce the edge energy.

Contact mode AFM was used to characterize the surface packing of bolaamphiphilic PDA ribbons on atomic level. The 2-D fast Fourier Transformation (2-D FFT) of scans over a flat ribbon surface suggests the formation of highly compact hexagonal packing arrangement of the polymer, with an approximate cell area of 20 $Å^2$, which is characteristic for tightly packed hydrocarbon chains. In contrast, earlier thermochromic studies on monofunctional PDA films using AFM showed that pseudo-rectangular packing arrangement was predominantly observed at room temperature for the blue phase film even when it was over-compressed during the preparation[25]. Our results demonstrate that the bolaamphiphilic lipid is able to form more stable and better-organized assemblies at ambient conditions. However, using this technique the distinction of the terminal carboxylate on the glutamate end from the one on the single carboxylate end would be difficult.

pH-induced optical and structural transformation in bolaamphiphilic PDA

A sharp blue-to-red color change was observed with Poly-*L*-Glu-Bis-3 upon the increase of pH. As expected, the existence of multiple base-sensitive carboxylic acid residues in the molecule resulted in a chromatic transition at a lower pH region compared to the poly-*L*-Glu-PDA assemblies discussed earlier. At pH 7.5, the blue polymer turned completely red as a result of significantly shortened conjugation length induced by the side chain disorder arising from increased electrostatic repulsion between deprotonated surface carboxylates.

Dramatic morphological changes accompanied the pH-induced colorimetric response of Poly-*L*-Glu-Bis-3 (Fig. 4). Extended ribbons were frayed into oriented nanofibers less than 10 nm in diameter upon the increase of pH. By exposing the polymer to more basic conditions for a longer time, more randomly coiled fibers were obtained. Figure 5 illustrates a proposed model of the transformation. Increased surface electrostatic repulsion upon the addition of base disrupts favorable H-bonding networks at the polar surface and overcomes

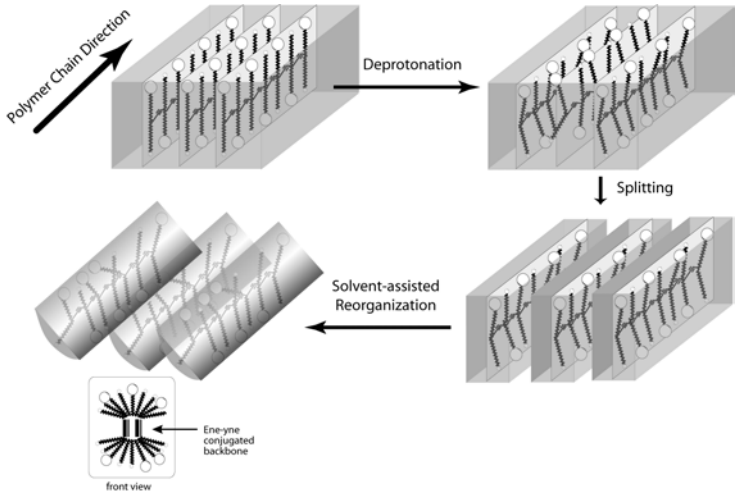


*Figure 5. A cartoon illustration of pH-triggered morphological transformation of **poly-L-Glu-Bis-3** from ribbons to nanofibers.*

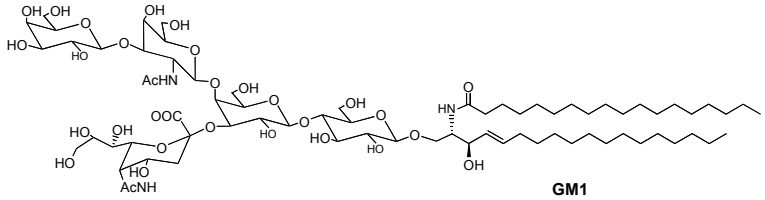
the attractive hydrophobic interactions between lipid cores, effectively splitting closely packed polymer chains into aligned fibers. The pH induced morphological transformation also reaffirms the linear propagation as the predominant format of polymerization of diacetylene units.

III. Lipid Doping-Induced Structural Transformation in Bolaamphiphilic PDA Assemblies

One fundamental consideration in designing biosensors is to establish an effective signal transduction pathway upon the interaction of incorporated receptors with analytes at the detection interface. For PDA-based colorimetric biosensors, this requirement transforms into a balance between the rigidity (which determines the extent of polymerization as well as the signal transduction efficiency) and flexibility (which is necessary for effective binding

of analytes with surface receptor as well as the lowering of transition energy barrier for the conformational change of the conjugation backbone) of the sensor scaffold. To strike a balance in the fluidity of the bolaamphiphilic PDA sensor scaffold, controlled doping with either the receptor as the only additive or by incorporating additional lipid dopants would be necessary. The diverse chemical structures of many naturally occurring lipids provide abundant possibilities to fine-tune the fluidity and morphological properties of bolaamphiphilic PDA-based biosensors.

Specifically, we are investigating the effect of lipid doping on the microstructural morphology of *L*-Glu-Bis-3 assemblies with the addition of G_{M1} ganglioside (structure shown below), a known receptor of cholera toxin, and/or cholesterol. Gangliosides are a family of glycosphingolipids localized to the outer leaflet of the plasma membrane of vertebrate cells. When inserted into



GM1

artificial membranes, the oligosaccharide motif of gangliosides is exposed at the membrane surface and functions as a recognition group for a number of bacterial toxins[16]. Polycyclic lipid cholesterol is known to directly participate in the formation of lipid rafts with glycosphingolipids. Both lipids have been shown to modulate domain structure and phase separation in model membrane systems[26].

Ribbon-to-vesicle microstructural transformation

When 5% G_{M1} ganglioside was introduced into the *L*-Glu-Bis-3 system, vesicles were formed along with ribbons (Fig. 6B). A significant number of vesicles, varied from less than 100 nm to greater than 500 nm in diameter, appeared to be attached to the ribbon structures, typically at the junction of several entangled ribbons. Incorporation of cholesterol at a low concentration (5%) along with G_{M1} led to the formation of an aggregate that allowed the development of a uniform blue color upon UV irradiation. However, the ternary system with high cholesterol content (20%) only led to the formation of turbid suspensions even after prolonged vortexing or probe sonication, leaving its photo-polymerizability at a minimum. TEM micrographs revealed that with increased cholesterol content, more vesicles were formed with continued coexistence of the ribbon structures (Fig. 6A). Apparently, addition of cholesterol further facilitates and stabilizes the formation of vesicles.

Cholesterol has long been known to stabilize membrane structures. Sphingolipids were thought to associate laterally with one another through

interactions between their headgroups, whereas cholesterol molecules function as spacers, filling the voids at the hydrophobic regions between associating sphingolipids. Such preferential packing was believed to lead to the formation

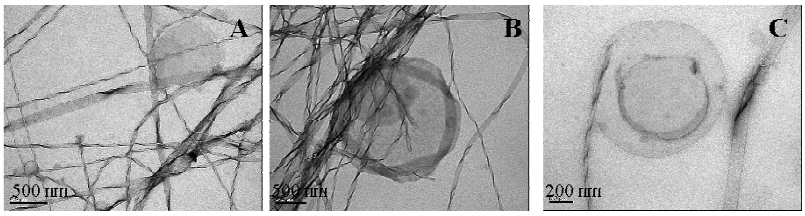


Figure 6. Transmission electron micrographs of L-Glu-Bis-3 doped with GM1 and cholesterol. (A) **L-*Glu-Bis-3*** *doped with 5% G_{M1} and 5% cholesterol; (B) doped with 5% G_{M1}; (C) doped with 5% G_{M1} and 10% cholesterol.*

of rafts within the membrane bilayers. In the three-component systems, we speculate that cholesterol molecules are inserted in the outer-surface of the vesicles, filling the voids at the hydrophobic region between aggregated gangliosides and membrane spanning lipids. Given the fact that none of G_{M1} ganglioside, cholesterol, or *L*-Glu-Bis-3 assembles by themselves to form vesicles, it is apparent that inserting the proper dopants between membrane spanning lipids is essential to inducing surface curvature and vesicle formation.

Some intriguing morphological details of doped *L*-Glu-Bis-3 assemblies captured by TEM (Fig. 6B,C) provided an opportunity to examine intermediate states of microstructural transformation between ribbons and vesicles. These micrographs clearly suggest that ribbons (relatively rigid) and vesicles (relatively fluid) were physically interrelated during the formation of different microstructures in these multi-component systems.

Morphological details of interconnected microstructures shown in Figures 6B (doped with 5% G_{M1}) and 6C (doped with 5% G_{M1} and 10% cholesterol), where the edges of vesicles or vesicle domains were outlined in the shape of ribbons, suggest a vesicle-to-ribbon transition mechanism at the periphery of vesicles. The growth of a ribbon and its extension away from a vesicular microstructure is most clearly seen in the image shown in Figure 6C. A vesicle-to-ribbon transition is a probable process during domain reorganizations within less crosslinked and more fluid vesicles. Lateral reorganization of lipids within these areas may have resulted in phases or domains with particularly low unpolymerizable dopant concentrations, thus a higher continuity of chirally packed matrix lipid *L*-Glu-Bis-3.

IV. Conclusions

Optically pure amino acid-terminated amphiphilic and bolaamphiphilic diacetylene lipids have been synthesized and assembled into tubes or ribbons with various dimensions and right-handed helicity. These microstructures can be photo-polymerized upon UV irradiation to form conjugated polymers with retained morphology and intense blue color, suggesting intrinsic highly ordered lipid packing arrangement and good alignment of diacetylene units that allow for topochemical polymerization. These conjugated polymer ribbons and tubes respond to external stimuli such as pH and heat via characteristic blue-to-red color change that is commonly observed with conventional thin film or vesicular PDA assemblies.

Careful chemical modifications made throughout the diacetylene lipid, including the installation of particular polar headgroup at either one end or both ends of the lipid, the selection of headgroup size, the manipulation of surface charge density as well as the positioning of the polymerization unit allow for the optimization of favorable inter-lipid interactions (e.g. H-bonding and *van der Waals* interaction) and the balance of dipolar forces that are critical to the formation of highly ordered supramolecular assemblies with unique microstructural morphology. Such rational design also brings control over the type and extent of optical and microstructural transitions of the material in response to specific external perturbation. In the case of Poly-*L*-Glu-Bis-3, the conjugated polymer responds to pH increase with a sharp colorimetric response as well as dramatic morphological changes from helical ribbons to aligned nanofibers.

In addition to physical environmental cues such as heat and pH, the incorporation of different lipid dopants into the bolaamphiphilic diacetylene assemblies also effectively induces microstructural transformations. Specifically, controlled doping of *L*-Glu-Bis3 with naturally occurring glycosphigolipid G_{M1} and cholesterol, which are relevant receptors for a number of potential biosensors, triggers the formation of fluid vesicles along with more crystalline ribbons. The multi-component system also allows for the observation of unique phase separation and microstructural transformaiton intermediates, resulting from dynamic clustering of the unpolymerizable lipid dopants and the reorganization of the polymerizable lipids in a relatively fluid environment.

Lessons learned from these studies provide valuable guidance to the rational design of future generations of PDA-based colorimetric sensors as well as other advanced nanomachinery where the microscopic morphology and optical properties of the material are crucial to its function.

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